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Topical Immunosuppressive drug combination roll-on for the treatment of Vitiligo: A promising approach

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ABSTRACT: Vitiligo is an inherited skin condition that affects 0.5% of the world's population and represents a significant dermatological health care issue. The condition, which is distinguished by patchy discoloration, is commonly linked to significant psychological suffering as well as aesthetic impairment. Genetic predisposition, inflammatory processes and oxidative stress are thought to be the primary pathophysiological factors for the depletion of functioning of melanocytes.

This is the inaugural trans-epidermal delivery of an epidermal melanocyte/keratinocyte solution from an autologous donor sample. The procedure appears to have several advantages since it is efficient, painless, non-invasive and efficacious in re-pigmenting vitiliginous skin by allowing the transplanted melanocytes to appropriately assimilate into the epidermal basal layer.

The goal of this initiative is to create multiple immunosuppressive medication mixtures which can be applied topically to alleviate vitiligo in its initial phases.

KEYWORDS: Genetic predisposition, inflammatory processes, betamethasone, tacrolimus, epidermal melanocyte.

AIM AND OBJECTIVES OF RESEARCH

Aim

The aim of formulating Betamethasone and Tacrolimus combination roll-on is to develop an effective and convenient topical medication for the treatment of acute vitiligo, which would lead to re-pigmentation of depigmented skin patches in affected individuals.

Objectives

- To determine the optimal concentration of Betamethasone and Tacrolimus combination for maximum efficacy in treating vitiligo.
- To develop a roll-on formulation of Betamethasone and Tacrolimus combination that provides easy and uniform application.
- To evaluate the stability of the roll-on formulation during storage and transport.
- To assess the safety and tolerability of the Betamethasone and Tacrolimus combination roll-on in human subjects.
- To investigate the re-pigmentation efficacy of the Betamethasone and Tacrolimus combination roll-on in patients with vitiligo.
- To compare the efficacy of the Betamethasone and Tacrolimus combination roll-on with other available treatment options for vitiligo.
- To prepare a comprehensive report on the formulation, characterization of the Betamethasone and Tacrolimus combination roll-on for the treatment of vitiligo

I. INTRODUCTION

The most typical reason for cutaneous depigmentation is a chronic condition called Vitiligo. Both sexes are equally impacted by the loss of functioning of t-lymphocytes in the cuticle and hair follicles, which typically begins somewhere between the age of 10 and 30. [1]

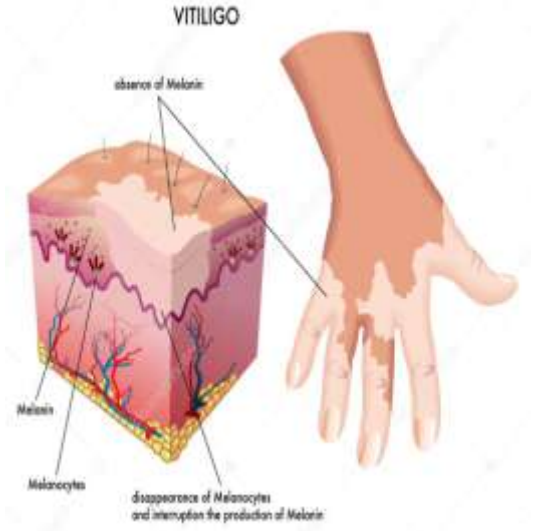

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Figure. 1: Anatomical skin representation of vitilgo and it's growth.

The exact pathology of vitiligo is unknown but four main theories exist to explain it are:

- The autoimmune hypothesis
- The neural hypothesis
- The self-destruction hypothesis
- The growth factor defect hypothesis.

Patients with vitiligo make up about 25% of those having a favorable family history. Moreover, more than 40 gene variations that generate innate immunity proteins or melanocyte constituents and are connected to the vitiligo phenotype have been found in genomic sequence association studies. [2]

There are several types of vitiligo, each with its own unique characteristics and patterns of depigmentation. Vitiligo can be classified into two major types such as non-segmental vitiligo and segmental vitiligo.

Non-segmental vitiligo: It is the most common type and typically affects both sides of the body symmetrically. It often begins as small, pale patches that gradually enlarge and spread over time. Non-segmental vitiligo can occur anywhere on the body, but it most commonly affects the face, hands, feet, and genital area. Within non-segmental vitiligo, there are further subtypes, including acrofacial vitiligo, which affects the fingers and face, and generalized vitiligo, which affects large areas of the body.

Segmental vitiligo: It is also known as unilateral or localized vitiligo, affects only one side of the body and tends to occur earlier in life than non-segmental vitiligo. It often appears as a single patch of depigmented skin, which may remain stable or spread over time. Segmental vitiligo is less common than non-segmental vitiligo, and it is often associated with less severe depigmentation.

Other less common types of vitiligo include mixed vitiligo, which is a combination of non-segmental and segmental vitiligo, and universal vitiligo, which affects nearly the entire body

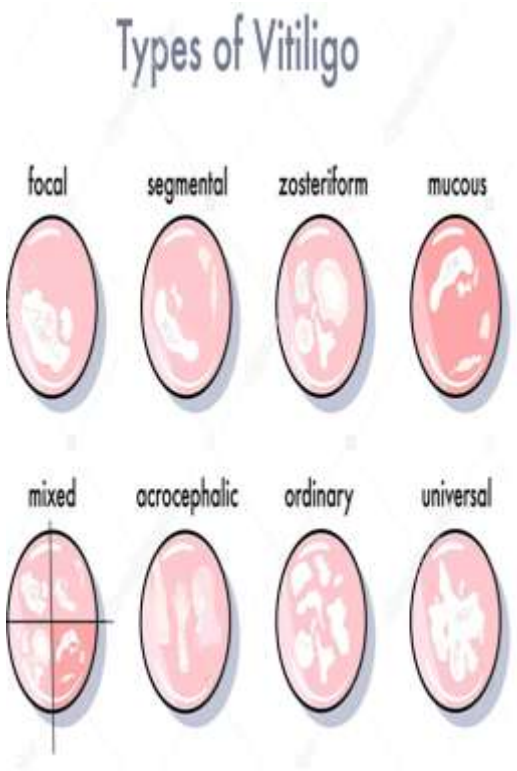

Figure. 2: Types of vitiligo on various skin areas

More and more data point to the fact that melanocytes are unable to respond to oxidative stress in a way that is acceptable. Reactive oxygen species (ROS), which are caused by both endogenous and external stimuli, are found in higher concentrations in afflicted individuals. Furthermore, antioxidant enzyme activity in the skin's epidermis is also seen to be reduced. [3]

The dermatomal pattern of vitiligo lesions and the idea that nerve endings release neurochemical mediators that are lethal to melanocytes explain the neurological explanation with reference to the pathophysiology of segmental vitiligo. This is corroborated by elevated levels of perilesional neuropeptide and the frequent occurrence of vitiligo after neurologic diseases. On the other hand, recent observational studies tend to imply a cutaneous mosaic and suggest segmental vitiligo is indeed a subvariant of non-segmental vitiligo. [4].

The several potential therapeutic options for vitiligo are challenging. Some of them are as follows:

- Several indigenous therapies
- Psoralens with laser therapy
- Topical and systematic corticosteroids

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- Tacrolimus
- Placental extract
- Cosmetic camouflaging
- Betamethasone

Various other therapies that are attempted with hope are the treatment of choice that are accessible. Getting a tattoo and skin grafting are additional alternative treatments.[5]

Betamethasone:

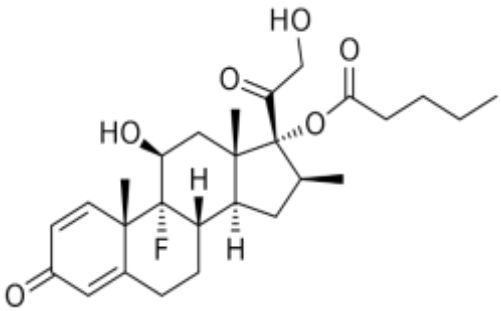

Figure 3: Structure of Betamethasone

Betamethasone is a potent synthetic corticosteroid that is used topically to treat various inflammatory skin conditions, such as eczema, psoriasis and dermatitis. It works by reducing inflammation, itching and redness in the affected area by suppressing the immune system's response and reducing the activity of certain chemicals that cause inflammation.

Betamethasone is available in several forms, including cream, lotion, and ointment. It should be applied thinly and evenly to the affected area once or twice a day, depending on the severity of the condition and the doctor's recommendation. It is important to follow the doctor's instructions carefully and not to use more than the prescribed amount, as excessive use of corticosteroids can lead to skin thinning, discoloration, and other side effects.

A strong topical corticosteroid with anti-inflammatory, anti-pruritic and vasoconstrictive properties is betamethasone. It results in delayed re-pigmentation, which can be accelerated by sunshine. When administered at large doses for an extended length of time under occlusive dressing, several adverse effects are observed. Dryness, itching, burning, localized irritability, telangiectasia, striae, skin atrophy, hyperkeratosis, alteration in coloration, secondary infection, blemish lesions and allergic dermatitis are all possible side effects of topical usage. Topical usage may result in systemic adverse effects such as hyperglycemia, HPA axis suppression, osteoporosis, muscular atrophy, Cushing's syndrome and growth suppression in adolescents.[5,6]

Tacrolimus:

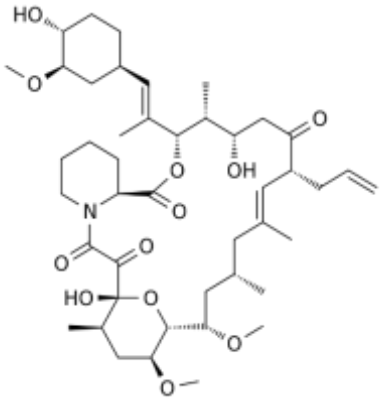

Figure. 4: Structure of Tacrolimus

Tacrolimus, is an immunosuppressive drug. Afterorgan transplant in humans, the risk of organ rejection is common. To decrease this risk of organ rejection, tacrolimus is given to the patient. Tacrolimus can also be prescribed for the treatment of T-cellmediated diseasessuch as eczema and psoriasis in the form of topical medication. For instance, it is prescribed for treating intensification of minimal change disease, serious refractory uveitis after a bone marrow transplant, Kimura's disease, and vitiligo. It can also be used to treat dry eye syndrome in pets.[5][6]

Tacrolimus obstruct calcineurin, which is associated in the production of interleukin-2, a molecule that contribute in the development and multiplication of T cells, as part of the body's learned (or adaptive) immune response.

Chemically, tacrolimus is a macrolide lactoneit was first discovered in 1987, from the fermentation broth of a Japanese soil sample that contained the bacterium Streptomyces tsukubensis. [7]

Tacrolimus is on the list of Essential Medicines of WHO

Usage of tacrolimus cream as a sole treatment for vitiligo. Tacrolimus 0.1% ointment used twice daily produced the best effects on lesions in the cephalic area, particularly the face. They block calcineurin activity, blocking the activation of T cells and the release of several inflammatory cytokines. The macrolide antibiotic

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tacrolimus, which is manufactured by Streptomyces tsukubaensis, has potent immunosuppressive and T-specific action.
Tacrolimus is commonly used in the prevention of transplant rejection after liver, kidney and heart transplantation. It is typically administered orally or topically, and like all immunosuppressant medications, it can have significant side effects and should only be used under the guidance of a healthcare professional. Some potential side effects of Tacrolimus include increased risk of infections, high blood pressure, and kidney damage.

NEED FOR THE RESEARCH WORK:

The need for the research work on developing a Betamethasone and Tacrolimus combination roll-on for the treatment of vitiligo is as follows:
1. Limited Treatment Options: There is a lack of effective treatment options for vitiligo, which is a skin condition that affects millions of people worldwide. Current treatments such as corticosteroids, immunosuppressants, and phototherapy have limitations in terms of efficacy, safety, and convenience.
2. Targeted Treatment: The Betamethasone and Tacrolimus combination roll-on provides a targeted approach to treat vitiligo by addressing the underlying immune dysfunction that causes the skin condition. The roll-on formulation provides an easy and uniform application, which may improve treatment adherence and efficacy.
3. Re-pigmentation: The Betamethasone and Tacrolimus combination has been shown to promote re-pigmentation in depigmented skin patches in patients with vitiligo. This is a significant improvement compared to current treatments that only aim to slow down or stop the progression of the condition.
4. Improved Safety Profile: The use of a topical Betamethasone and Tacrolimus combination roll-on may have a better safety profile compared to systemic treatments such as corticosteroids and immunosuppressants. The roll-on formulation may also reduce the risk of skin atrophy and other adverse effects associated with long-term use of corticosteroids.
5. Clinical Need: There is a significant clinical need for an effective and safe treatment for vitiligo, which can improve the quality of life of affected individuals. The development of a Betamethasone and Tacrolimus combination roll-on may address this clinical need and provide a new treatment option for vitiligo.

II. METHODOLOGY

Ingredients and their Role

Chemicals	Role
Betamethasone	Immunosuppressant
Tacrolimus	Immunosuppressant
Benzyl Alcohol	Preservative
Menthol	Cooling And Emollient Property
PEG400	Penetration Enhancer
Isopropyl Myristate	Moisturizer And Thickening Agents
Triethanolamine	pH Adjusting agent
Orange Peel Oil	Fragrance
Ethanol	Vehicle

Table 1:Ingredients and their Role

- Betamethasone is a type of corticosteroid medication. Corticosteroids are a class of drugs that mimic the effects of cortisol, a hormone that is naturally produced by the adrenal

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glands. They have anti-inflammatory and immunosuppressive properties and are commonly used to treat a wide range of conditions, including allergic reactions, asthma, autoimmune disorders, and certain types of cancer. [7]

- Tacrolimus is an immunosuppressant medication. It belongs to a class of drugs called calcineurin inhibitors and is used to prevent the rejection of transplanted organs or tissues by suppressing the immune system. [8]

- Benzyl alcohol is a type of preservative that is commonly used in pharmaceuticals, cosmetics, and personal care products. It is a colorless liquid with a mild, pleasant aroma and is often used as an alternative to other preservatives that may be harsh or irritating to the skin. In pharmaceuticals, benzyl alcohol is often used as a preservative to prevent the growth of bacteria and other microorganisms in multi-dose vials of injectable medications. It is also used as a solvent in some medications and as a local anesthetic. [9]

- Menthol is a compound derived from the peppermint plant that is commonly used in cosmetics and personal care products for its cooling and emollient properties. When applied to the skin, menthol creates a cooling sensation by activating the body's cold receptors. This can provide relief from minor skin irritations and itching, and it can also help to soothe sore muscles and joints. [10]

- Polyethylene glycol 400 (PEG 400) is a type of polyethylene glycol that is commonly used in pharmaceuticals as an emulsifier and conditioning agent. As an emulsifier, PEG 400 is used to help mix two or more liquids that would otherwise be immiscible, such as oil and water. It can improve the stability and consistency of emulsions, suspensions, and other types of liquid formulations. As a conditioning agent, PEG 400 is used to help moisturize and soften the skin. It can be found in topical medications such as ointments and creams, and it is also used in some oral medications to improve their taste and texture. [11]
- Isopropyl myristate is a type of fatty acid ester that is commonly used in pharmaceuticals, cosmetics, and personal care products as a moisturizer and thickening agent. As a moisturizer, isopropyl myristate helps to hydrate and soften the skin by forming a protective barrier on its surface. This can help to prevent dryness and improve the overall texture and appearance of the skin. As a thickening agent, isopropyl myristate is used to increase the viscosity of liquid formulations, such as lotions, creams, and ointments. It can also improve the spreadability and absorption of these formulations, making them easier to apply and more effective at delivering their active ingredients. [12]
- Triethanolamine (TEA) is a common ingredient in pharmaceuticals and personal care products, where it is primarily used as a pH adjuster. As a pH adjuster, TEA is used to control the acidity or alkalinity of a formulation. It is often used in topical medications, such as creams and ointments, to adjust the pH to a level that is compatible with the skin's natural pH. This can help to prevent irritation and improve the effectiveness of the medication. [13]
- Ethanol, also known as ethyl alcohol, is a common solvent and vehicle used in pharmaceuticals. It is a clear, colourless liquid that is miscible with water and many organic solvents, making it a versatile ingredient for a wide range of pharmaceutical formulations. Ethanol is often used as a base or vehicle for oral liquid medications, such as cough syrups, as it helps to dissolve active ingredients and improve their palatability. It is also used in topical medications, such as gels and creams, to help solubilize active ingredients and improve their absorption into the skin. [14]

FORMULATION

For 10ml (Each 1 ml contains)

Chemicals	F1	F2	F3
Betamethasone	1mg	1mg	1mg
Tacrolimus	1mg	1mg	1mg

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Benzyl Alcohol	0.05ml	0.05ml	0.05ml
Menthol	23mg	23mg	25mg
PEG400	0.1ml	0.1ml	0.1ml
Isopropyl Myristate	0.1ml	0.1ml	0.1ml
Triethanolamine	0.2ml	0.2ml	-
Orange Peel Oil	0.01ml	0.01ml	0.01ml
Ethanol	-	q.s.	q.s.
Water	q.s.	-	-

Table 2: Formulation

METHOD OF PREPARATION:

Step 1:

• Since ethanol mixes well with API and other excipients, it was employed in this formulation as the solvent.

• In beakers 1 and 2, respectively, a homogeneous solution of tacrolimus and betamethasone was made.

the final solution was replenished with triethanolamine (if required to adjust the pH) and benzyl alcohol.

• In beaker 3, isopropyl myristate and PEG 400 were combined to create a dense phase solution.

Step 2:

• Menthol was dissolved in ethanol before being added to beaker 1, which was followed by the step-by-step addition of the contents of beakers 2 and 3.

Step 3:• As intended,

Figure 5: Sonication of the final solution

CHALLENGES AND SOLUTIONS:

1. The partial solubility of the active components in the water in F1 affected the compatibility with other excipients. Hence, the formulation uses ethanol as the basic medium to address the aforementioned problem.
2. Agglomeration and precipitation of white constituents occur when Triethanolamine, a stabilizer and pH adjuster, is added to the formulation, which results in formulation instability.
3. In F2 the roll on cooling nature and emollient action was not appropriate.

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4. In F3 the quantity of menthol was adjusted accordingly and the pH of the formulation was as per the pH of the skin.

Figure 6: Agglomeration of white constituent

EVALUATION TEST

1. Organoleptic Evaluation

I. Colour

The final formulation was having pale yellow colour

Figure 7: Final formulation in measuring cylinder

II. **Odour**

Pleasant smell of orange peel

2. pH Parameter

The pH of formulated roll on was determined using pH paper and also by pH meter. [15,16]

By pH paper-5

By pH meter- 5.53

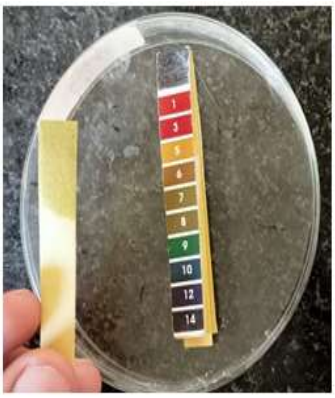

Figure 8: pH determination

3. Spreadability

The two slides may separate from roll-ons and move into one another under the influence of a specific load, the faster is the Spreadability. Spreadability is measured in seconds. The faster the spreadability, the more slides can move into one another.

It is calculated by using formula

S= M.L/T

Where, M= Weight tied to upper lid,

L= Length of glass slides,

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T= Time taken to separate the slides

S= M.L/T

S= 0.5× 7.5/30

S= 3.75/30

S= 0.125

Spreadability = 0.125cm/ml/sec

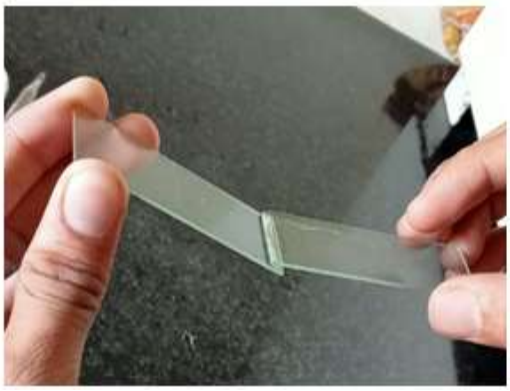

Figure 9: Spreadability testing using glass slides

5. Drying time

Roll-on antiperspirants and liquid deodorants should swiftly evaporate from the skin's surface following application. If consumers do not wait until the product is totally dry on their skin, the slow drying period will result in a sticky feeling and may leave stains on the garments. Stickiness is a factor that can be used to judge drying time.

When 0.5ml of roll on was applied on the skin under room temperature, drying time was found to be 30 seconds.

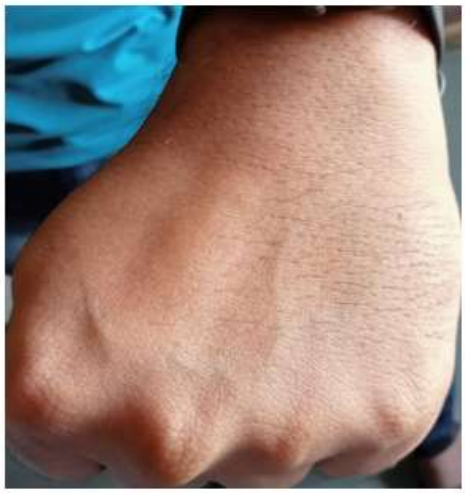

Figure 10: Drying time determination

5. Skin irritating test

It is carried out by applying product on the skin for 30 min. [17]

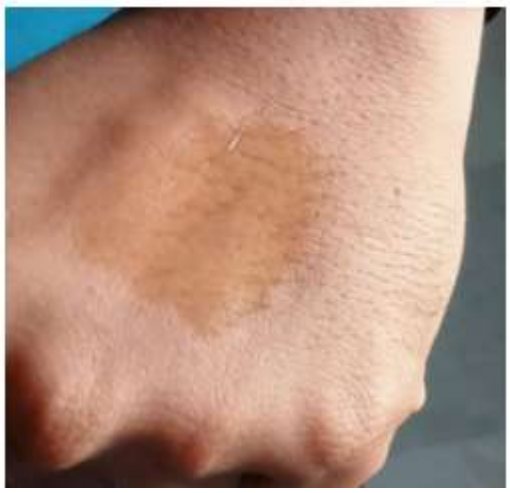

Figure 11: Formulation applied foe determination of skin irritation

6. Viscosity

The viscosity of roll is calculated by Ostwald's viscometer.

Water

T1 = A to B = 1.57min

T2 = A to B = 1.58min

T3= A to B = 1.59 min

Mean = t1 + t2 + t3/3

= 1.57+1.58+1.59/3

= 1.58min

1min= 60 sec

1.58min = x

X = 1.58×60/1

X = 94.8 sec

Sample

T1= 7.40 min

T2= 7.41 min

T3= 7.40 min

Mean= t1+t2+t3/3

= 7.40+7.41+7.40/3

= 7.40min

1min= 60sec

7.40min= x

X= 444sec

Density determination

Wt of empty bottle specific gravity (w1) = 12.19g

Wt of bottle+ water (w2) = 20.41g

Wt of bottle+ sample (w3) = 19.35g

Density of sample = W3-W1/W2-w1

Density = 19.35-12.19/20.41-12.19

= 0.8710g/ml

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Viscosity of liquid = p2t2/p1t1× n1

= 0.8710× 444/1× 94.8× 0.997

= 386.724/94.8× 0.997

= 4.079× 0.997

Viscosity = 4.066 cp

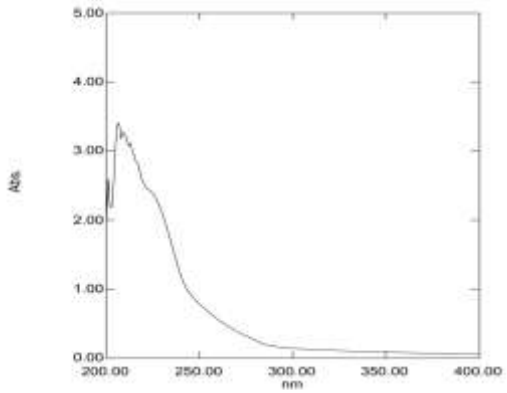
Figure 12: Ostwald viscometer

Result of Evaluation Tests:

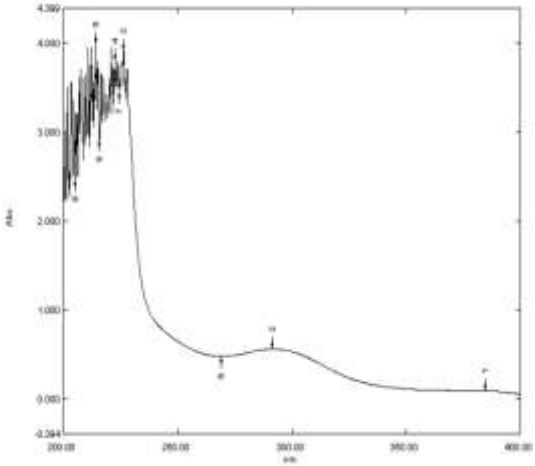
Evaluation parameter	**Inference**
Colour	Pale yellow
Odour	Orange peel
pH	5
Viscosity	4.066 cp
Spreadability	0.125cm/ml/sec
Drying time	30 sec
Skin irritating test	No irritation

7. Absorption spectra of individual API

1. Betamethasone Lambda max (λmax) 249nm



2. Tacrolimus Lambda max (λmax) 291 nm



8. Franz Diffusion Test:

The Franz diffusion experiment is a widely used method to determine the drug release and permeation characteristics of topical formulations. The procedure for the Franz diffusion experiment of the Betamethasone and Tacrolimus Combination roll-on is as follows:

Equipment and Materials:

- Franz diffusion cell with a receptor compartment capacity of 15-20 mL
- Outer thin egg membrane with a pore size to permeate solution
- Magnetic stirrer
- Syringe filters (0.45 µm)
- Betamethasone and Tacrolimus Combination roll-on
- Phosphate buffer saline (pH 7.4)
- Analytical balance
- Ultraviolet-visible spectrophotometer

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Procedure:

1. The Franz diffusion cell is assembled by placing the thin egg membrane between the donor and receptor compartments.
2. The receptor compartment is filled with 15-20 mL of phosphate buffer saline (pH 7.4) and placed on a magnetic stirrer.
3. The donor compartment is loaded with 5 ml of the Betamethasone and Tacrolimus Combination roll-on.
4. The donor compartment is sealed with parafilm to prevent evaporation and maintain the temperature at 32°C ± 0.5°C.
5. At predetermined time intervals (15 min), 5 mL of the receptor fluid is withdrawn and replaced with fresh buffer.
6. The collected samples are filtered through 0.45 µm syringe filters and analyzed using an ultraviolet-visible spectrophotometer to determine the amount of Betamethasone and Tacrolimus that permeated through the thin egg membrane.
7. The experiment is continued until steady-state conditions are achieved, typically for 1 hour.
8. The permeation profile is then constructed by plotting the cumulative amount of drug permeated against time.

Figure. 13: Prepared Phosphate Buffer 7.4

Figure. 14: Set up of Franz diffusion

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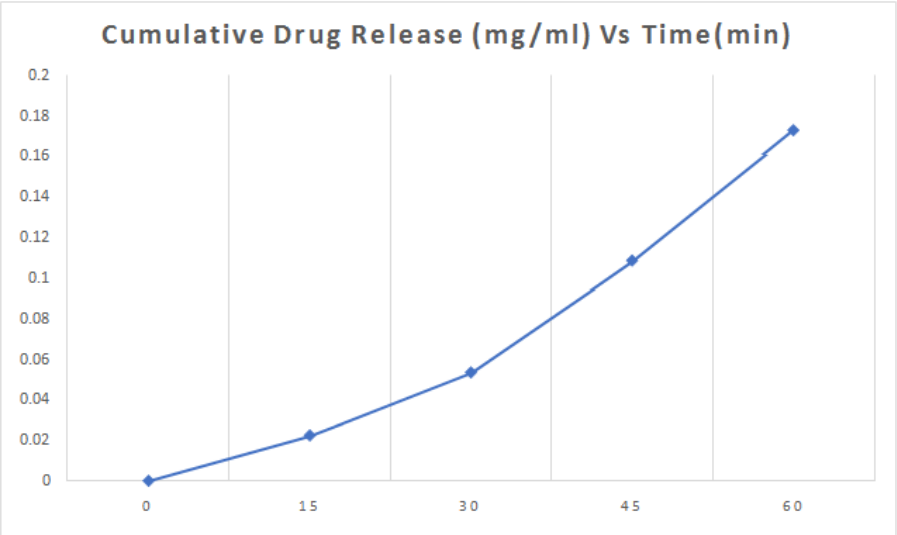
Result of Franz Diffusion

1. Drug Release of Betamethasone

Time(Min)	Abs at Wl 249.0 nm
0	0
15	0.323
30	0.449
45	0.797
60	0.928

Table 3: Betamethasone Drug release data



Graph 1: Cumulative Drug Release Vs Time For Betamethasone

2. Drug Release of Tacrolimus

Time (Min)	Abs at Wl 291.0 nm
0	0
15	0.12
30	0.13
45	0.149
60	0.162

Table 4: Tacrolimus Drug Release data

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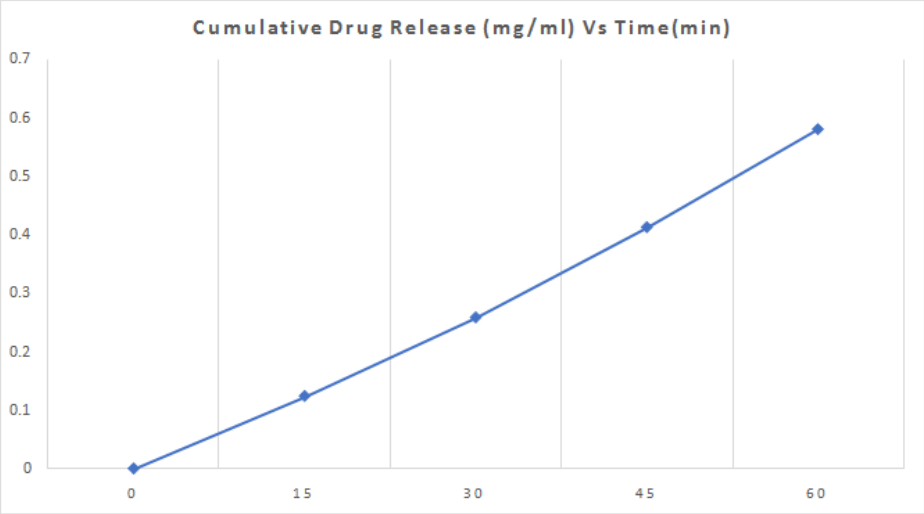

Graph 2: Cumulative Drug Release Vs Time For Tacrolimus

III. RESULT AND DISCUSSION

The evaluation studies for the roll-on revealed that the formulation was clear and homogeneous. The pH was almost near to the pH of the skin.

The organoleptic test results showed that the prepared formulation had a pleasant odor and was easily applicable.

The pH of the formulation was found to be within the range suitable for skin application.

The drug content of the prepared formulation was found to be within the acceptable range determined using a UV-visible spectrophotometer.

The skin irritation test showed that the formulation did not cause any skin irritation.

The skin penetration and Franz diffusion studies showed that the prepared formulation had good skin penetration.

The viscosity of the formulation was found to be appropriate for easy application using a roll-on applicator.

IV. CONCLUSION

As there is always an increasing demand for patient convenience and compliance related research, among the various route of administration, the topical route is the most popular route for administration of therapeutic agents because of the ease of administration and also because of its low costs of therapy.

The study successfully formulated and evaluated a roll-on containing betamethasone and tacrolimus drug with ethanol as a base the treatment of vitiligo. The prepared formulation showed good physical and chemical properties, including appropriate color, odor, consistency, pH, drug content and viscosity. The formulation also exhibited good skin penetration, sustained drug release and no skin irritation, suggesting its potential for use as a treatment for vitiligo. However, further studies are required to evaluate the long-term stability and efficacy of the prepared formulation.

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